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Novel Methodology for the Extraction and Quantitative Analysis of Calcium Sennosides in Senna Leaves

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ARTICLE DETAILS

Research Paper

Keywords:

Thermosil C18 column, Calcium Sennosides , RP-HPLC,UV, Senna

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ABSTRACT

The developed method was for the Extraction and Quantitative Analysis of Calcium Sennosides in Senna Leaves. From literature review and solubility analysis initial chromatographic conditions Mobile phases methanol: water 40:60 were set symmetry C 18.1 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 255 nm. The retention times for calcium sennosides was found to be 12 min and quantitative analysis calculated for HPLC and UV compared with standard and the sample Senna and the extract was found to be 20 % and Practical yield of 27 grams and the yield was found to be 2.7 % by taking 1 kg of Senna leaves and the pH adjusted with ammonium Hydroxide to 6.0 to 6.5.

Introduction

Senna leaves come from the Senna plant (Cassia senna), a tropical shrub native to Africa, Asia, and the Middle East. The leaves have been used for centuries in traditional medicine, particularly in Ayurvedic and Unani practices. The Active compounds are Anthraquinone glycosides (sennosides A and B). Calcium sennosides are a crucial component of Senna leaves, responsible for their medicinal properties. Here are some key importance of calcium sennosides:

Laxative effect: Calcium sennosides stimulate bowel movements, relieving constipation and promoting regularity. Purgative properties: They help eliminate toxins and waste from the body, supporting detoxification. Anti-inflammatory: Calcium sennosides exhibit anti-inflammatory properties, soothing irritated tissues and reducing swelling. Antimicrobial: They possess antimicrobial properties, inhibiting the growth of harmful bacteria and fungi. Antioxidant: Calcium sennosides have antioxidant properties, protecting against oxidative stress and cell damage.

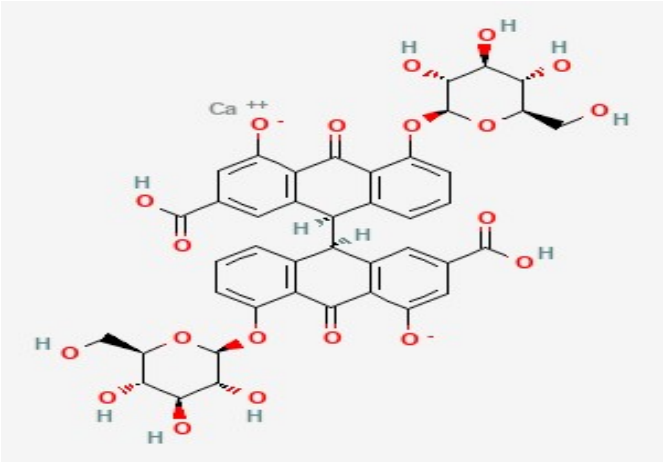


Fig no :1 Structure of Calcium Sennosides

Chemicals and Reagents:

Water [HPLC Grade], Acetonitrile [HPLC Grade], Rifampicin [Working standards], Potassium Dihydrogen Phosphate & ortho phosphoric acid ,Ammonium Hydroxide ,90 % Ethanol ,Concentrated HCl ,Anhydrous calcium chloride ,Muslin Cloth all the chemicals were procured from standard solutions and the tablets were collected from the Local market.

Instrumentation:

Chemicals & Reagents:

Calcium sennosides Standard 20 % Extract (Himalayan Nutraceuticals Pvt Ltd) and pure samples will be extracted with ammonium Hydroxide by adjusting the pH 6.0 to 6.5 and 1 Kg of Senna leaves purchased from Rhutvik Enterprises.
HPLC grade and AR grade solvents will be purchased.

Instrument details:

- UV Spectrophotometer: Thermo Fisher Scientific, Model-GEN10S UV-Vis. Software- blank
- HPLC: Shimadzu, Model- Shimadzu SPD-M20A 230V, BRISA LC2 C18 5µm 25×0.46 column, Software – LC-Solution
- Sonicator: ANTech (GTsonic), Model – GT -1730QTS.
- Analytical Balance: WENSAR, Model- 75533 IND/09/08/466 PGB200
- pH Meter: HANNA, Model- pH211
- Magnetic Stirrer: REMI, Model- 1-MLH
- Hot Air oven: SINGHLA

Chromatographic Condition:

The mobile phase includes 40:60 methanol: water and Ortho phosphoric acid was pre-owned for pH adjustment of buffer to 3. The mobile phase was strained through 0.45 µm membrane filter and degassed by sonication. The flow rate was set to 1.0ml/min. The drug shows good absorbance at 254 nm, which was selected as wavelength for further analysis.

Preparation of Mobile Phase: To establish a relevant HPLC method, couple of different mobile phases were attempted. Decided based on the readily available solvents, short run time, results and sensitivity of assay. Mobile phases concluded here were aqueous solvent, i.e., MilliQ water and an organic solvent methanol. Solvents were filtered separately through 0.45 µm membrane filter and degassed by sonication.

Preparation of Standard solution: 10 mg of the Calcium sennosides was weighed accurately and was dissolved in 100 mL methanol separately 100 µg/mL. It was sonicated to dissolve completely. Then it was filtered through membrane filter paper. This standard stock solution used to prepare necessary concentrations to construct calibration curve by proper dilution.

Preparation of Sample solutions: The extracted Senna leaves 10 mg taken and transferred in to 100 mL light resistant flask and made up to the required volume by using mobile phase. The solution was filtered through 0.45 µm filter. From the filtrate 10 mL was taken separately in a 25 mL volumetric flask and a sample solution concentration of 100 µg/ml.

Procedure of extraction

Take 1 Kg of Senna Leaves in a tall Stainless steel vessel. Add 90 % Ethanol to the brim and after 4 Hours add Concentrated HCl and adjust the pH to 2.5, then filter through a muslin cloth. To the filtrate add a calculated quantity of anhydrous calcium chloride. Adjust the pH with ammonium hydroxide to 6.0 to 6.5 Calcium Sennoside gets precipitated. Filter and collect the precipitate which contains calcium sennosides. The Filtrate obtained can be used for next batch.

RESULTS AND DISCUSSIONS

Calculation:

Practical Yield (%) = (0.2 grams10 grams) ×100

This method ensures you provide a clear and precise calculation of the practical yield for the extraction and estimation of calcium sennosides from Senna leaves.

Practical Yield (%) = (Initial Weight of Senna Leaf Powder / Weight of Calcium Sennosides obtained) ×100

1Kg of Senna leaves yield 7 grams of Sennosides that means contains 0.7 % of Sennosides

$$\%\text{Yield} = (0.1/\ 180) \times 100$$

$$= 0.09\%$$

Given:

Practical yield = 27grams

Quantity of Senna leaves = 1000grams

Percentage yield = 27/ 1000 ×100

=2.7%

To calculate the quantitative analysis of Calcium Sennosides using HPLC, follow these steps:

1. Prepare the standard solution: Prepare a standard solution of Calcium Sennosides with a known concentration (usually 100 µg/mL).
2. Inject the standard solution: Inject the standard solution into the HPLC system and record the peak area.
3. Determine the response factor (RF): Determine the response factor (RF) using the formula:

RF = (Peak area of standard solution x Concentration of standard solution) / (Amount of standard solution injected)

1. Prepare the sample solution: Prepare the sample solution by dissolving the Calcium Sennosides powder in a solvent (usually methanol or water).
2. Inject the sample solution: Inject the sample solution into the HPLC system and record the peak area.
3. Calculate the concentration of the sample solution: Calculate the concentration of the sample solution using the formula:

Concentration of sample solution (µg/mL) = (Peak area of sample solution x RF) / (Amount of sample solution injected)

1. Calculate the quantity of Calcium Sennosides: Calculate the quantity of Calcium Sennosides in the sample using the formula:

Quantity of Calcium Sennosides (mg) = (Concentration of sample solution x Volume of sample solution) / 1000.

Table no: 1 Calculations of Calcium sennosides for HPLC

Standard concentration = 100 ppm
Sample concentration= 100 ppm

Standard area=700069
Sample area= 698557
Standard dilution= 10/100
Sample dilution= 10/100
Potency= 20

Calculations:

$$\% = \frac{\text{SAMPLE AREA}}{\text{STANDARD AREA}} \times \frac{\text{STANDARD DILUTION}}{\text{SAMPLE DILUTION}} \times \frac{\text{POTENCY}}{100} \times \frac{100}{LABEL\ CLAIM}$$

$$= \frac{698557}{700069} \times \frac{10}{100} \times \frac{100}{10} \times \frac{20}{100} \times \frac{100}{1}$$

$$= 19.94\%$$

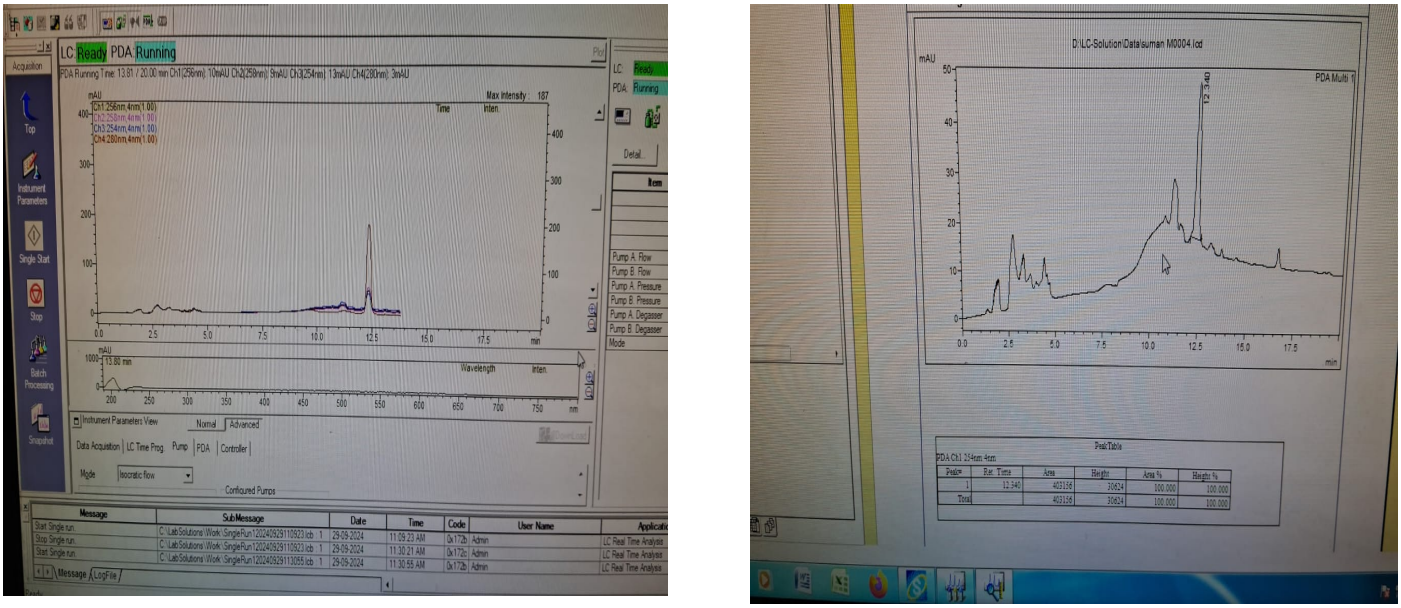


Figure no :1 Retention time of HPLC Calcium Sennosides 12 minutes

To calculate the quantitative analysis of Calcium Sennosides using UV spectroscopy, follow these steps:

1. Prepare the standard solution: Prepare a standard solution of Calcium Sennosides with a known concentration (usually 100 µg/mL).
2. Measure the absorbance of the standard solution: Measure the absorbance of the standard solution at the specified wavelength (usually 254 nm or 340 nm).
3. Determine the extinction coefficient (E): Determine the extinction coefficient (E) using the formula:

E = (Absorbance of standard solution x Dilution factor) / (Concentration of standard solution x Path length)

1. Prepare the sample solution: Prepare the sample solution by dissolving the Calcium Sennosides powder in a solvent (usually methanol or water).
2. Measure the absorbance of the sample solution: Measure the absorbance of the sample solution at the same wavelength as the standard solution.
3. Calculate the concentration of the sample solution: Calculate the concentration of the sample solution using the formula:

Concentration of sample solution (µg/mL) = (Absorbance of sample solution x Dilution factor) / (E x Path length)

1. Calculate the quantity of Calcium Sennosides: Calculate the quantity of Calcium Sennosides in the sample using the formula:

Quantity of Calcium Sennosides (mg) = (Concentration of sample solution x Volume of sample solution) / 1000.

Calcium Sennosides by UV:

Calculation:

Standard Solution:

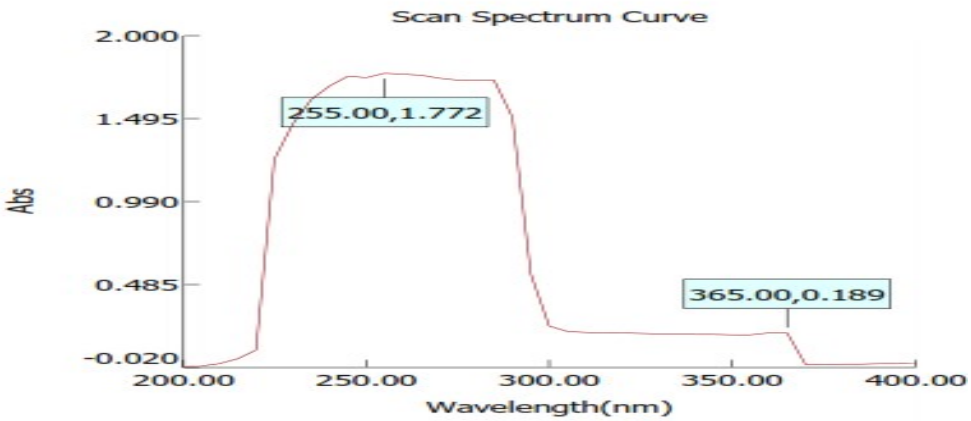
$$E = \frac{\text{Standard Absorbance X Dilution Factor}}{\text{Concentration of Standard Solution x Path Length}}$$

$$= \frac{0.080 \times 10/100}{100 \times 1} = 0.00008$$

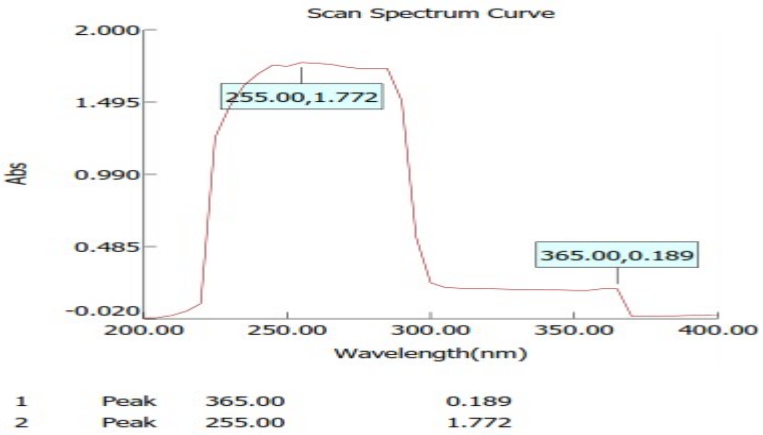
Sample Solution:

$$\text{Concentration of Sample Solution (ppm)} = \frac{\text{Absorbance of Sample Solution X Dilution Factor}}{E \times \text{Path Length}}$$

$$= \frac{0.0160 \times 10/100}{0.00008 \times 1} = 20\ \%$$



1	Peak	365.00	0.189
2	Peak	255.00	1.772



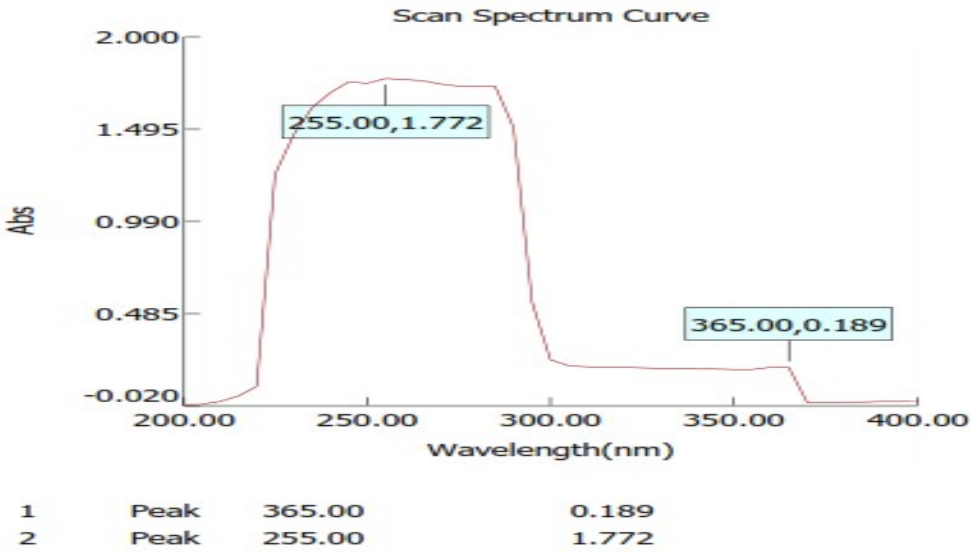


Figure no :1 Lamda max for Calcium sennosides at 255 nm

Table no :2 The retention time of Calcium Sennosides was found to be 12 mins

S. No	Name of compound	Amount taken(mg)	%Purity
1	Calcium Sennosides HPLC	10 mg	`19.94 %

2	Calcium Sennosides UV	10 mg	20 %

Conclusion

It is concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the analysis of Calcium Sennosides with less tailing factor and is also economical. Thermosil RPC18 (4.5×100nm) 5.0 µm, flow rate was 1ml/min. Both samples in the range of 200 to 400 nm and maximum wavelength was identified at 255 nm.Extraction of calcium sennosides and the yield calculated and found to be 2.7 %

CONSENT AND ETHICAL APPROVAL

It is not applicable

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COMPETING INTEREST

Authors have declared that no completing interests exists

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